APPLICATION INVOLVING RHIZOBIUM AND AZOTOBACTER

Week 1 (2015/06/21-2015/06/27)

1. Bacteria transformation for phosphate sensor testing

Week 2 (2015/07/19-2015/07/25)

1. Made media for rhizobium and azotobacter Tx pSH033 Cl into *E. coli* XL1 EC

2. Made 1 L TY Media

3. Made 500 mL YMB Media

4. Made 500 mL of Azotobacter Media

5. Made 16 plates of Azotobacter Media

6. Made 16 plates of YMA

7. Transformed pSH033 Cl plasmid into E. coli XL1 EC.

8. Ramya plated 100 microliters and 100 microliters of 1:100 dilution on LB/Chlor(17) plates. Incubated at 37 C

9. Grew glycerol stock of Rhizobium on toothpick in 3 mL YMB at RT and 250 rpm for 3 days.

10. Transformed pSH033 into S17 E. coli Heat Shock Competent Cells by heat shock protocol. Incubated 1 h at 250 rpm and 37C in 1 mL SOC and plated 100 microliters and the rest on LB/Chl(17) plates.

11. 1 Colony on LB/Chl(17) plate with 900 microliters.

Week 3 (2015/07/26-2015/08/01)

1. Grew cultures of pSH033 in XL1 EC (2), pSH033 + pET28a in SH17 (2), and pSH033 in SH17 (from the plate transformed on July 24th) in 3 mL LB/Chl(17).

2. Streaked rhizobia on plate and put in incubator at 27 C for 3 days.

3. Cultures of pSH033 in XL1 EC (2) grew, but cultures of pSH033 + pET28a in SH17 (2), and pSH033 in SH17 (from the plate transformed on July 24th) did not grow.

4. Miniprepped pSH033 in XL1 EC and measured concentrations by nanodrop. (QIAGEN Miniprep Kit)

5. Made glycerol stocks (500 microliters 50% glycerol and 500 microliters culture) of pSH033 in XL1 EC and rhizobia. Put in -80C box

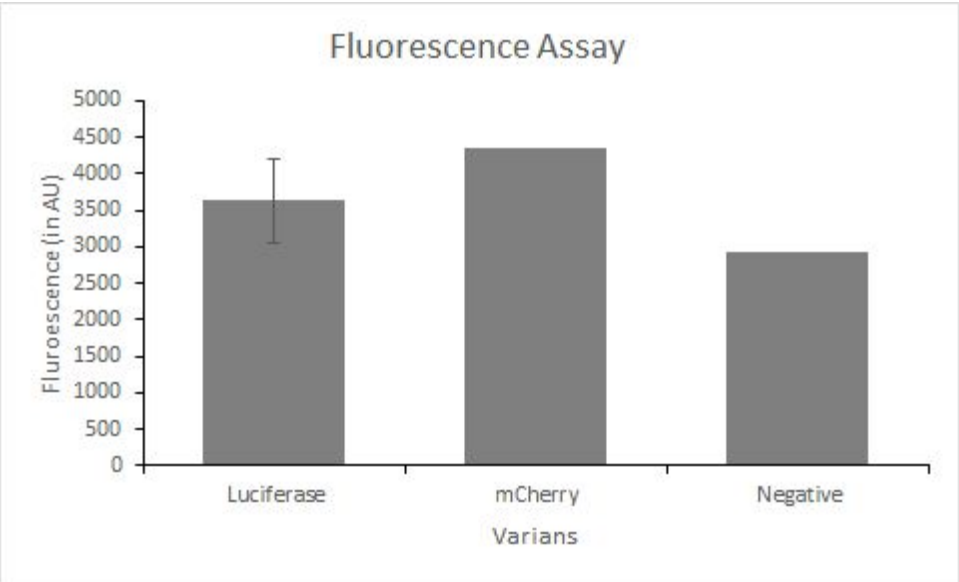
6. Streaked LB plate with frozen SH17 (glycerol stock) to make heat shock competent cells and Incubated 22 h at 37 C.

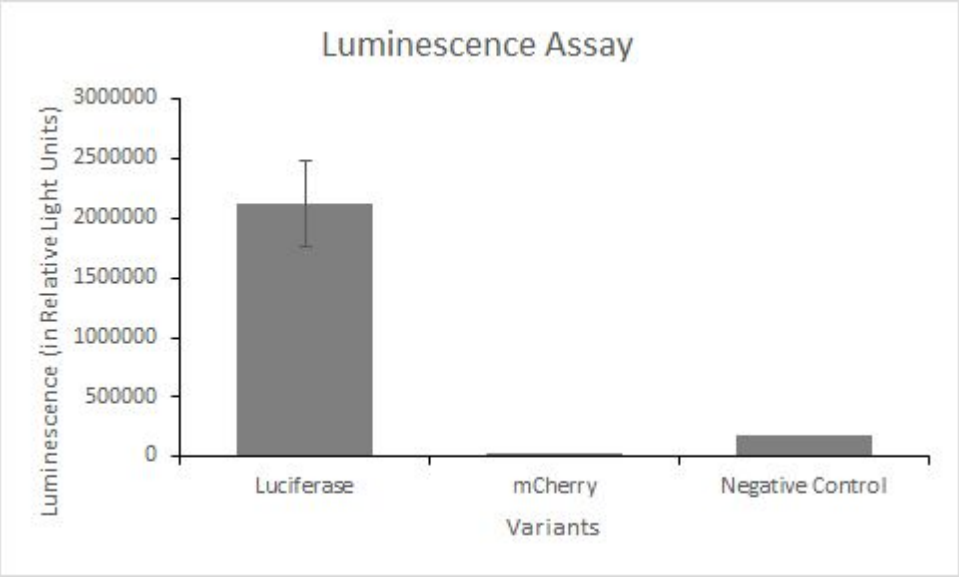
7. Made heat shock competent S17 E.coli cells

8. Grew 3 mL LB/Chl(34) pSH033 in S17. Grew 3 mL LB/Tet(10) pAM120 in S17 from 9:15 A.M. at 37C, 250 rpm.

9. What I need to do next time: Conjugation of rhizobia and E. coli cells

10. Transcribing and translating mCherry without cells





Week 4 (2015/08/23-2015/08/29)

1. Grew rhizobia in 5 mL of YMB at 27C. (4 culture tubes for a total of 20 mL)

2. Autoclaved small pieces of filter paper in aluminum foil in jar (usually the jar holds

toothpicks). Used vacuum setting 1.

3. Streaked culture of MG1655 E. coli on LB plate. Incubated 18 h at 37C.

4. Designed primers to get pSH033 in pSB1C3.

5. Reconstituted Azotobacter. Heated glass. Added water until glass cracked.

6. Filed off glass. Removed cotton plug with tweezers in ethanol.

7. Added 1 mL Azotobacter supplement to pellet. Transferred pellet to tube.

8. Incubated 4 mL Azotobacter + supplement in tube at 250 rpm and 27C. 5 P.M.

9. Streaked two plates of Azotobacter. Incubated at 27C. Made two sets of glycerol stocks. Stored in -80C in Matt Bennett's lab.

10. Grew E. coli MG1655 in 5 mL LB culture tubes. Incubated at 250 rpm, and 37C. 8:15 P.M.

11. Started 5 mL LB culture of pSH033 in S17 (Chl17) and 5 mL culture of pAM120 in S17 (Tet 5). Incubated at 250 rpm and 37C. 10:10 A.M.

12. Checked OD600 at 1:30 P.M. 0.012 :(

13. Checked OD600 at 7 P.M. :(

14. Started new cultures as stated in #1 except using Tet 2.5 @ 7 P.M.

15. Checked OD 600 at 10:00 A.M. 24 hr growth = 4, 15 hr growth 1 for pSH033 in S17.

a. No growth in pAM120 in S17.

16. Started 2 new 5 mL cultures of pSH033 with OD600 of 0.1352 in 500 microliter culture

17. Incubated at 37C, 250 rpm. After 1 h, checked OD → 0.4-0.5.

18. Started conjugation of rhizobia and pSH033 S17 E. coli.

19. Repeated #18 except with 5 mL of MG1655 E. coli instead of rhizobia.

20. Started conjugation of rhizobia and pSH033 S17 E. coli.

21. Repeated #20 except with 5 mL of MG 1655 E. coli instead of rhizobia and put on LB plate.

22. Made 1 L of YMA. Autoclaved on setting 3 (liquids).

23. Made YMA/Strep(50)/Chl(17), YMA/Strep(50), and YMA/Chl(17).

24. Took out 1.5 mL centrifuge tubes and plates after 19 h.

25. Plated 100 microliter of straight, 1:10, 1:100, and 1:1000 dliutions of Rhizobia +

S17 E. coli on YMA/Spec(50)/Chl(17). Repeated with MG1655 E.coli and S17 E. coli except plated 1:100 and 1:1000 on LB/Chl(17) plates.

26. Put LB plates in 37 C. Put YMA plates in 30 C.

27. Put 10 microliters of Azotobacter culture into 3 mL of AZ Supplement, 3 mL YMB, and 3 mL LB. Put 10 microliters of rhizobia only culture in 3 mL YMB. Shook at 250 rpm and room temperature.

28. Streaked Rhizobia + S17 E. coli on YMA/Chl(17) plate. Streaked rhizobia on YMA/Spec(50) plate. Streaked azotobacter on AZ plate and YMA plate. Put all plates in 30C incubator.

29. Made left over azotobacter into glycerol stock.

Week 5 (2015/08/30-2015/09/05)

1. Took out plates with Azotobacter only.

2. Colonies started to form on plates with conjugation.

3. Started to PCR conjugation factor out of pSH033 using Phusion.

4. Ran 0.8% agarose gel in TAE to verify size.

5. Digested PCR product with Dpn1 for 1 h at 37C.